

1 **Title page**

2 **An outbreak of *Streptococcus pyogenes* in a mental health facility: Advantage of**
3 **well-timed Whole Genome Sequencing over *emm* typing.**

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38 **Summary** (40 words)

39 When controlling an outbreak of *S. pyogenes* mediated by dermal colonization in a
40 mental health facility, Whole Genome Sequencing (WGS) provided data in a
41 timeframe sufficient to guide infection control measures and identified person-to-
42 person transmissions better than *emm* typing.

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51 **Abstract:**

52 **Background:** In June 2016 an outbreak control team was formed following

53 notification of four cases of *Streptococcus pyogenes* in a residential ward in a large

54 mental health facility.

55 **Methods:** Active surveillance was conducted to identify new cases of *S. pyogenes* and

56 targeted epidemiological investigations and infection control measures were

57 implemented. Single nucleotide polymorphism (SNP) based genome phylogeny, *emm*

58 typing and multilocus sequence typing (MLST) were performed on the *S. pyogenes*

59 isolates. We compared the ability of WGS and *emm* typing to correctly identify

60 person-to-person transmission and guide the management of the outbreak.

61 **Results:** 204 patients and 152 staff members were investigated. We identified 35

62 patients and two staff members with *S. pyogenes*. WGS revealed polyclonal *S.*

63 *pyogenes* infections with three genetically distinct phylogenetic clusters (C1-C3).

64 Cluster C1 isolates were all *emm* type 4, sequence type 915 and had pairwise SNP

65 difference of 0-4 which suggested recent person-to-person transmissions.

66 Epidemiological investigation revealed that Cluster C1 was mediated by dermal

67 colonization and transmission of *S. pyogenes* in a male residential ward. Clusters C2

68 and C3 were genomically more diverse with pairwise SNP differences of 21-45 and

69 26-58 and *emm* types 11 and mostly *emm*120 respectively. Clusters C2 and C3, which

70 may have been considered person-to-person transmissions by *emm* typing, was shown

71 by WGS to be unlikely by integrating pairwise SNP differences with epidemiology.

72 **Conclusion:** WGS had higher resolution than *emm* typing in identifying clusters with

73 recent and ongoing person-to-person transmissions, which allowed implementation of

74 targeted intervention to control the outbreak.

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Introduction:

Streptococcus pyogenes is a human pathogen causing a range of illnesses from pharyngitis and impetigo to necrotizing fasciitis and streptococcal toxic shock syndrome. The epithelial surfaces of the throat and skin are the principal sites of asymptomatic *S. pyogenes* colonization and the sites for most new *S. pyogenes* acquisitions and transmissions [1, 2].

Healthcare-associated outbreaks of invasive *S. pyogenes* infections in both acute care [3-5] and long-term care facilities [6-12] are well described. Between 5-12% of cases of severe *S. pyogenes* infection are found to be healthcare-associated [1, 4, 5]. The control of outbreaks in both acute and long-term care facilities is challenging, partly due to patients' underlying medical conditions and behaviours.

In June 2016, *S. pyogenes* was isolated from four patients within (one bacteraemia and three wound infections) from a male residential ward (Ward A) in a mental health facility in Singapore. Since this was a surge from the baseline incident cases of *S. pyogenes* (1-2 cases over the previous 6 months), an institutional-wide outbreak investigation was initiated. This facility is a 2,000-bed tertiary psychiatric hospital providing acute and chronic mental health services. It is situated on a large open plan campus, which includes 50 inpatient wards, seven specialist outpatient clinics and long-term residential care units, including assisted living quarters for patients with chronic mental health issues.

An outbreak control team was convened to determine the extent and epidemiology of the outbreak, to identify potential breaches in infection control practice and to provide recommendations to prevent further transmission of infection. As classical *emm* typing for *S. pyogenes* was not available in Singapore, the outbreak control team decided whole genome sequencing (WGS) would be used as the primary

typing method to assist traditional epidemiology during this investigation. Here, we report the utility of WGS in guiding the outbreak management and compare its performance with *emm* typing.

Methods:

Definitions

Cases included patients and staff with *S. pyogenes* infection and/or asymptomatic throat or skin carriage between 01/06/2016 and 31/12/2016. Invasive disease was defined as the isolation of *S. pyogenes* from normally sterile sites. Community isolates were those with no known epidemiological link to the institution where the outbreak occurred. These isolates were collected from a different healthcare institution between November 2015 and April 2016.

Case finding

To identify cases, we collected oropharyngeal swabs from residents and staff members on the affected wards and swabs from residents with visible wounds on skin. Staff members were questioned regarding signs and symptoms of *S. pyogenes* infection in the month prior to identification of the index cases. Medical records were reviewed to track the movements of patients and staff across the hospital in order to identify possible transmission routes.

Laboratory investigation of isolates

Samples taken from patients and staff members at the outbreak institution were processed remotely at the Department of Laboratory Medicine in Tan Tock Seng Hospital. The samples were cultured to detect *S. pyogenes* using standard methods (see Supplementary Methods). Cultured organisms were identified using MALDI TOF (Bruker, Bremen, Germany). Antimicrobial susceptibility testing was performed

using the disc diffusion method as per Clinical and Laboratory Standards Institute (M100-S25) [13]. *emm* typing was retrospectively performed using PCR and Sanger sequencing as described by the Centres for Disease Control and Prevention (CDC; <http://www.cdc.gov/ncidod/biotech/strep/protocols.html>) and *emm* types assigned using the CDC database (<http://www2a.cdc.gov/ncidod/biotech/strepblast.asp>).

WGS of *S. pyogenes* isolates

WGS of samples took place on three occasions between 17/06/2016 and 18/10/2016 at the Genome Institute of Singapore. Genomic DNA was extracted from 40 isolates from patients and staff from the affected institution and 24 randomly selected community derived *S. pyogenes* isolates. The 24 community isolates (GAS001-024) provided background genetic information on *S. pyogenes* circulating locally. We performed single nucleotide polymorphism (SNP) based genome phylogeny (using genomic regions that are devoid of mobile genetic elements), *emm* typing and multilocus sequence typing (MLST) on the *S. pyogenes* isolates (see Supplementary Methods). Clusters of closely related strains were identified by manual inspection of the phylogenetic tree; these are referred to as “genomic clusters” in the rest of the manuscript. Sequencing files (Fastq) were submitted to the GenBank Sequence Read Archive (SRA) under study accession number SRP111309.

The outbreak control team determined the presence of person-to-person transmissions of *S. pyogenes* (directly or indirectly) based on integrating data from epidemiological investigations combined with pairwise SNP differences (from WGS) and *emm* types. Comparison was made between WGS and *emm* typing in supporting the correct identification of person-to-person transmissions and guiding the management of the outbreak.

Results

As part of the outbreak investigation, 204 patients and 152 staff members were tested for *S. pyogenes* infection or colonisation between 1st June and 31st December 2016. *S. pyogenes* was isolated from 35 patients (17% of all patients screened) in eight wards and two (1.3%) staff members. Four affected patients required hospitalization in another medical facility. Two of the four patients had bacteraemia. One of the bacteraemic patients had necrotising fasciitis and required below knee amputation while the other was diagnosed with a psoas abscess. One patient had groin cellulitis requiring intravenous antibiotics and the fourth patient required incision and drainage of a finger pulp abscess. Most patients had superficial skin infections. All patients recovered. Characteristics of all the affected wards and infection control interventions are described in supplementary methods.

Microbiological results

All *S. pyogenes* isolates from the affected institution were susceptible to penicillin, erythromycin and clindamycin and resistant to tetracycline. *Staphylococcus aureus* was also isolated with *S. pyogenes* from many of the swabs taken from skin lesions. These *S. aureus* isolates displayed many different antibiograms on susceptibility testing and were not typed further as part of this investigation (see Supplementary Results).

Epidemiologic and genomic investigation of the outbreak

WGS was performed on 64 *S. pyogenes* isolates (see Supplementary Results). Three patients' samples had not been sequenced at the time of writing. Sequencing identified 16 *emm* types which matched the *emm* types determined by Sanger sequencing (see Supplementary Results). Sixteen different MLST types were determined including seven novel sequence types. Novel MLST types were submitted to pubmlst.net. The new MLST types determined were ST547, ST909, ST915-ST919

(Figure 1). Manual examination of strain genetic relatedness identified three genomic clusters (C1-C3) (Figure 1). Based on pairwise SNP differences, Cluster C1 contained the most closely related strains and was the top candidate cluster for recent person-to-person transmissions of *S. pyogenes*.

Cluster C1 included isolates from 16 patients from Ward A, two patients from Ward C, one patient from Ward D and one community isolate, GAS008. Ward A was the site of initial temporal cluster of 4 infections that prompted investigation in June 2016. Infections from this cluster occurred intermittently from June to the end of October 2016 (Figure 2).

Wards A and C are male residential wards for those with chronic mental health issues. On investigation, the majority (>80%) of Ward A patients suffered with xerosis with histories of recurring superficial skin infections (Supplemental material section 4) Most residents had frequent physical contacts during their daily activities. Ward C was adjacent to Ward A, and patients from these two wards shared communal living and dining areas where they spent most of their time taking part in planned social activities. Most patients in Ward C had no chronic skin lesions. Ward D patients were mainly undergoing psychiatric rehabilitation which included interacting with patients in other wards. These patients were independent in their activities of daily living with no history of skin infections or chronic skin lesions. We could not establish an epidemiological link between patients from Ward A, C and Ward D by assessing patient movement, staff cross-coverage, and shared activities. All patients and staff on these wards were negative for throat carriage of *S. pyogenes*. No link could be established between the community isolate GAS008 and the affected patients in the institution.

The results of WGS showed that the *S. pyogenes* isolates from June (Wards A and C) and July, August, and October (Wards A,C, and D) clustered tightly on the phylogenetic tree (Cluster C1) and were genetically distinct from most of the background community isolates (GAS001-GAS024) (Figure 1). Cluster C1 isolates had pairwise SNP difference of 0-4 and were *emm* type 4 and ST915. The low pairwise SNP distances was consistent with recent transmission of *S. pyogenes* among the patients involved in this cluster.

Cluster C2 contains isolates from five patients from Ward B, one patient each from Wards G and H and a community isolate, GAS022 (Figure1). All Ward B isolates were collected in July following a case of invasive infection while isolates from Ward G and H were obtained in September (Figure 2). Oropharyngeal carriage of *S pyogenes* was identified in one staff member (STAFF 1) who worked on Ward B. This triggered a separate epidemiological investigation (see Supplementary Material). No epidemiological link could be established between Ward B and Wards G and H or the community isolate (GAS022). There were no further cases related to Cluster C2 after September 2016. The *S. pyogenes* strains in Cluster C2 were all *emm* type 11 and ST-547. However, they were genetically more divergent than those in Cluster C1 with pairwise SNP differences of 21-45, which is suggestive of a more distant common ancestor for this population and therefore independently introduced infections rather than recent transmissions in the wards. Additionally, the STAFF 1 isolate was genetically distinct from Cluster C2 patients' isolates.

The *S. pyogenes* isolates in **Cluster C3** had pairwise SNP difference of 26-58. The outbreak control team therefore considered Cluster C3 not to have arisen from recent person-to-person transmissions and likely to represent sporadic cases of infection with common community *S. pyogenes* strains.

Practicalities of WGS and its influence on infection prevention and control measures

The outbreak control team decided on the timing of WGS depending on the number and characteristics of affected patients, number of involved staff members and the geographical distribution of the affected wards. The results of WGS guided specific infection control interventions at different points in time over the investigation period as described in Table 1. The outbreak peaked between September and October and settled by November 2016 (Figure 3).

The average cost of WGS over the outbreak period was USD 220 per isolate with a minimum turnaround time of eight days. Once established at the department of Laboratory Medicine in Tan Tock Seng Hospital the cost of *emm* typing by Sanger sequencing was USD 146 per isolate with a turnaround time of three days.

Discussion

WGS generated typing data in a clinically relevant time frame for the management of this outbreak. Additionally, it provided greater resolution compared to *emm* typing in identifying a cluster with ongoing transmissions.

Skin carriage acted as the main reservoir for *S. pyogenes* in Cluster C1 with none of the patients having throat colonisation. Many of the affected patients had multiple risk factors for skin infection including residing in a tropical climate, crowded living conditions, previous scabies infections and breaks in the skin. Additionally, prolonged and close direct skin contact was common among the residents [2]. All Cluster C1 isolates were of *emm* type 4, which accounts for 14% of *emm* types circulating in Asia [14] and belongs to the *emm* E pattern group. The *emm* pattern genotype is used as a marker for tissue site tropism of *S. pyogenes*

strains. Patterns A to C are associated with throat infections; pattern D are considered skin specialists and pattern E strains are considered generalists and are associated with either throat or skin infection [14-16]. A dermatologist was appointed to the institution and individual patient skin care plans were implemented (supplemental material section 4). This led to an improvement in the patients' skin condition with no further *S. pyogenes* infections in the index ward. Serious consideration was given to the use of institution-wide antibiotic treatment for *S. pyogenes* with the discovery of multi-ward involvement of the *S. pyogenes* infections. This intervention has previously been described to control outbreaks of *S. pyogenes* in long-term care facilities [6, 11, 17]. Given the numbers of patients and staff members in the institution (at least 3000) the cost, logistics, complexity and possible adverse effects of this intervention would have been significant. Fortunately, WGS and epidemiology enabled us to differentiate the one cluster with recent person-to-person transmission (Cluster C1) from the two other clusters which were sporadically introduced infections without recent transmission (Clusters C2-C3). This information allowed outbreak control interventions to be ward and patient-based rather than institution-wide (Table 1). It also avoided unnecessary ward closures and restrictions on patients' movement that would have disrupted the normal functioning of the facility for both inpatients and outpatients.

We concluded that the cases in Cluster C1 constituted an outbreak of a single clone of *S. pyogenes*, which is supported by the strong epidemiological connection (nearly all from Ward A), identical *emm* types, ST types, antibiotic resistance profiles and very close genome sequences (pairwise distances of 0-4). Similar data (concordant epidemiology and pairwise differences of < 14 SNPs) has been used previously to support the conclusion of clonal outbreaks of *S. pyogenes* causing lethal

puerperal sepsis [18], direct transmission between two closely related care homes [19] and *emm59* invasive disease [20]. The strains in Cluster C2-C3 also had identical *emm* types (except for C3 wherein there was 1 strain with different *emm* type), ST types and antibiotic resistance patterns, but had higher pairwise SNP differences, i.e., 21-45 for C2 and 26-58 for C3 respectively. Moreover, for Clusters C3, there was little epidemiological connection between the patients. Higher pairwise SNP differences along with epidemiological data allowed us to preclude any person-to-person transmissions in the Clusters C2-C3. Importantly, based on *emm* typing alone without WGS, we would have considered intra-ward and inter-ward transmission for these clusters. Similar conclusions have been drawn in reports of *S. pyogenes* infections with similar SNP differences. For example, Tagini et al. ruled out clonal outbreaks of invasive infections caused by *S. pyogenes* with the same *emm* type and ST type in Switzerland but with pairwise differences of 14-32 SNPs between these strains [21]. Indeed, the genomic mutation rate of *S. pyogenes* has been estimated at 1.3-2.1 SNP /strain/year [22-24] suggesting that clusters with SNP differences in the range of that seen in Switzerland or greater (as in Cluster C2 and C3) may be independent non-outbreak infections by closely related (and probably locally circulating) *S. pyogenes* strains.

While we define Cluster C1 as an outbreak cluster because of very low SNP differences (0-4 SNPs) between the isolates and define Clusters C2-C3 as non-outbreak clusters due to higher SNP differences, it should be emphasized that using mere SNP counts as a proxy to help delineate clonal spread should be treated with caution. We believe there are confounding variables that pose difficulties in imposing a simple threshold for the number of SNPs between isolates to decide if they are part of a recent transmission event. Koser et al. describe the presence of a hypermutator

phenotype in an MRSA isolate from an outbreak in a neonatal unit which resulted in that isolate having a higher number of SNPs than the other outbreak isolates [25]. Other factors that may affect SNP differences include differing rates of accumulation of genome polymorphisms among *S. pyogenes* strains over time and organism population size [26]. We also relied on a reference-based analysis to calculate SNP distances; this would not capture differences in many mobile genetic elements, which might demonstrate higher SNP differences [27]. Furthermore, the effect of environmental conditions, tissue site, or presence of other organisms (such as *S. aureus*) on the mutation rate is unknown.

emm typing, which involves sequencing of the short, hypervariable region at the 5' end of *emm* gene is the most commonly used *S. pyogenes* typing method in outbreak investigations [28, 29]. Sanger-based *emm* typing was performed in the laboratory at Tan Tock Seng Hospital to corroborate the WGS derived *emm* types (see Supplementary Results). In retrospect, given the particular distribution of *emm* types amongst the institution and community isolates in this outbreak, *emm* typing would have identified the polyclonal nature of the infections, however Clusters C2 and C3 may have been wrongly classified as a transmission clusters if only *emm* typing was used. The greater discriminatory power of WGS allowed detection of the recent transmission events in Cluster C1 and the non-clonal nature of Clusters C2 and C3. Certainly, WGS would have been critical to determine the relatedness of isolates if all isolates have had the same *emm* type [19, 29, 30].

In future outbreaks of *S. pyogenes*, WGS should be considered the primary typing method to guide the outbreak management if rapid access to the technology is available. However, if WGS is not immediately available, a two-step approach with an initial traditional *emm* typing followed by WGS to further discriminate closely

related isolates, would be a reasonable.

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References

1. Lamagni TL, Darenberg J, Luca-Harari B, et al. Epidemiology of severe *Streptococcus pyogenes* disease in Europe. *Journal of clinical microbiology* **2008**; 46(7): 2359-67.
2. Walker MJ, Barnett TC, McArthur JD, et al. Disease manifestations and pathogenic mechanisms of Group A *Streptococcus*. *Clin Microbiol Rev* **2014**; 27(2): 264-301.
3. Daneman N, McGeer A, Low DE, et al. Hospital-acquired invasive group a streptococcal infections in Ontario, Canada, 1992-2000. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **2005**; 41(3): 334-42.
4. Kakis A, Gibbs L, Eguia J, et al. An outbreak of group A Streptococcal infection among health care workers. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **2002**; 35(11): 1353-9.
5. Steer JA, Lamagni T, Healy B, et al. Guidelines for prevention and control of group A streptococcal infection in acute healthcare and maternity settings in the UK. *The Journal of infection* **2012**; 64(1): 1-18.

- 349 6. Auerbach SB, Schwartz B, Williams D, et al. Outbreak of invasive group A
350 streptococcal infections in a nursing home. Lessons on prevention and control.
351 Archives of internal medicine **1992**; 152(5): 1017-22.
- 352 7. Davies HD, McGeer A, Schwartz B, et al. Invasive group A streptococcal
353 infections in Ontario, Canada. Ontario Group A Streptococcal Study Group.
354 The New England journal of medicine **1996**; 335(8): 547-54.
- 355 8. Harkness GA, Bentley DW, Mottley M, Lee J. Streptococcus pyogenes
356 outbreak in a long-term care facility. American journal of infection control
357 **1992**; 20(3): 142-8.
- 358 9. Ruben FL, Norden CW, Heisler B, Korica Y. An outbreak of Streptococcus
359 pyogenes infections in a nursing home. Annals of internal medicine **1984**;
360 101(4): 494-6.
- 361 10. Schwartz B, Elliott JA, Butler JC, et al. Clusters of invasive group A
362 streptococcal infections in family, hospital, and nursing home settings.
363 Clinical infectious diseases : an official publication of the Infectious Diseases
364 Society of America **1992**; 15(2): 277-84.
- 365 11. Smith A, Li A, Tolomeo O, Tyrrell GJ, Jamieson F, Fisman D. Mass antibiotic
366 treatment for group A streptococcus outbreaks in two long-term care facilities.
367 Emerging infectious diseases **2003**; 9(10): 1260-5.
- 368 12. Thigpen MC, Richards CL, Jr., Lynfield R, et al. Invasive group A
369 streptococcal infection in older adults in long-term care facilities and the
370 community, United States, 1998-2003. Emerging infectious diseases **2007**;
371 13(12): 1852-9.

- 372 13. Clinical and Laboratory Standards Institute (M100-S25). Performance
373 Standards for Antimicrobial Susceptibility Testing: Twenty Fifth
374 Informational Supplement. **January 2015**.
- 375 14. Steer AC, Law I, Matatolu L, Beall BW, Carapetis JR. Global emm type
376 distribution of group A streptococci: systematic review and implications for
377 vaccine development. *The Lancet Infectious diseases* **2009**; 9(10): 611-6.
- 378 15. McGregor KF, Spratt BG, Kalia A, et al. Multilocus sequence typing of
379 *Streptococcus pyogenes* representing most known emm types and distinctions
380 among subpopulation genetic structures. *J Bacteriol* **2004**; 186(13): 4285-94.
- 381 16. Tewodros W, Kronvall G. M protein gene (emm type) analysis of group A
382 beta-hemolytic streptococci from Ethiopia reveals unique patterns. *Journal of*
383 *clinical microbiology* **2005**; 43(9): 4369-76.
- 384 17. Jordan HT, Richards CL, Jr., Burton DC, Thigpen MC, Van Beneden CA.
385 Group a streptococcal disease in long-term care facilities: descriptive
386 epidemiology and potential control measures. *Clinical infectious diseases* : an
387 official publication of the Infectious Diseases Society of America **2007**; 45(6):
388 742-52.
- 389 18. Turner CE, Dryden M, Holden MT, et al. Molecular analysis of an outbreak of
390 lethal postpartum sepsis caused by *Streptococcus pyogenes*. *Journal of clinical*
391 *microbiology* **2013**; 51(7): 2089-95.
- 392 19. Chalker VJ, Smith A, Al-Shahib A, et al. Integration of Genomic and Other
393 Epidemiologic Data to Investigate and Control a Cross-Institutional Outbreak
394 of *Streptococcus pyogenes*. *Emerging infectious diseases* **2016**; 22(6): 973-80.

- 395 20. Engelthaler DM, Valentine M, Bowers J, et al. Hypervirulent emm59 Clone in
396 Invasive Group A Streptococcus Outbreak, Southwestern United States.
397 Emerging infectious diseases **2016**; 22(4): 734-8.
- 398 21. Tagini F, Aubert B, Troillet N, et al. Importance of whole genome sequencing
399 for the assessment of outbreaks in diagnostic laboratories: analysis of a case
400 series of invasive Streptococcus pyogenes infections. European journal of
401 clinical microbiology & infectious diseases : official publication of the
402 European Society of Clinical Microbiology **2017**; 36(7): 1173-80.
- 403 22. Beres SB, Carroll RK, Shea PR, et al. Molecular complexity of successive
404 bacterial epidemics deconvoluted by comparative pathogenomics. Proc Natl
405 Acad Sci U S A **2010**; 107(9): 4371-6.
- 406 23. Nasser W, Beres SB, Olsen RJ, et al. Evolutionary pathway to increased
407 virulence and epidemic group A Streptococcus disease derived from 3,615
408 genome sequences. Proc Natl Acad Sci U S A **2014**; 111(17): E1768-76.
- 409 24. Turner CE, Abbott J, Lamagni T, et al. Emergence of a New Highly
410 Successful Acapsular Group A Streptococcus Clade of Genotype emm89 in
411 the United Kingdom. mBio **2015**; 6(4): e00622.
- 412 25. Koser CU, Holden MT, Ellington MJ, et al. Rapid whole-genome sequencing
413 for investigation of a neonatal MRSA outbreak. The New England journal of
414 medicine **2012**; 366(24): 2267-75.
- 415 26. Fittipaldi N, Beres SB, Olsen RJ, et al. Full-genome dissection of an epidemic
416 of severe invasive disease caused by a hypervirulent, recently emerged clone
417 of group A Streptococcus. Am J Pathol **2012**; 180(4): 1522-34.
- 418 27. Feil EJ, Holmes EC, Bessen DE, et al. Recombination within natural
419 populations of pathogenic bacteria: short-term empirical estimates and long-

420 term phylogenetic consequences. Proc Natl Acad Sci U S A **2001**; 98(1): 182-
421 7.

422 28. Athey TB, Teatero S, Li A, Marchand-Austin A, Beall BW, Fittipaldi N.
423 Deriving group A Streptococcus typing information from short-read whole-
424 genome sequencing data. Journal of clinical microbiology **2014**; 52(6): 1871-
425 6.

426 29. Facklam R, Beall B, Efstratiou A, et al. emm typing and validation of
427 provisional M types for group A streptococci. Emerging infectious diseases
428 **1999**; 5(2): 247-53.

429 30. Ben Zakour NL, Venturini C, Beatson SA, Walker MJ. Analysis of a
430 Streptococcus pyogenes puerperal sepsis cluster by use of whole-genome
431 sequencing. Journal of clinical microbiology **2012**; 50(7): 2224-8.
432

Table 1. Influence of whole genome sequencing (WGS) results on infection prevention and control measures

Phase 1 (2nd-11th June)	Pre-WGS	Post-WGS	Remarks
<p>Strains sent for WGS: 17th June 2016</p> <p>WGS results: 8th July 2016</p>	<p>Summary:</p> <ol style="list-style-type: none"> 1. There were potential transmissions of <i>S. pyogenes</i> involving wards A and C. 2. <i>S. pyogenes</i> colonisation and transmission was probably mediated by chronic skin lesions. 3. Staff, visitors and volunteers were ruled out as potential sources of infection as their throat swabs were all negative. 4. Infection control recommendations were prescribed as per supplementary Table 1 and Figure 1. 	<p>WGS results:</p> <ol style="list-style-type: none"> 1. Isolates from wards A and C were genetically linked (Figure 1-Cluster 1). 2. Pairwise SNP distances suggested transmissions involving wards A and C. 3. The pairwise SNP distances also suggested this strain may have been circulating for approximately 2 years 	<ol style="list-style-type: none"> 1. WGS confirmed person-to-person transmission of <i>S. pyogenes</i> involving Wards A and C. 2. WGS results did not significantly change the outbreak management at this time

	<p><i>Pre-WGS hypotheses and planning parameters</i></p> <ol style="list-style-type: none"> 1. A typing method was needed to define if it was large cluster of genetically linked <i>S. pyogenes</i> isolates or multiple small clusters. 2. WGS is accepted as a typing methods by the outbreak control team. 	<p><i>Post-WGS conclusions and recommendations by the outbreak control team</i></p> <ol style="list-style-type: none"> 1. Ward A appears to be the epicentre of the outbreak, most likely due to the extensive chronic skin lesions observed among most patients and patients' psycho-social behaviour patterns. 2. Ward C is potentially involved due to the extensive physical contacts between patients from wards A and C in the common areas. 3. To continue the focus on patient and environmental hygiene measures to prevent further transmission of GAS. 4. Patient referral to external dermatologist 	
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		<p>for management of skin conditions.</p> <p>5. Enhanced hospital-wide surveillance for skin lesions. If present, to screen for GAS by wound culture.</p>	
<p>Phase 2 (27th July-31st August)</p>	<p>Pre-WGS</p>	<p>Post-WGS</p>	<p>Remarks</p>
<p>Strains sent for WGS: 19th August 2016</p> <p>WGS results: 5th September 2016</p>	<p>Summary:</p> <ol style="list-style-type: none"> 1. More cases of <i>S. pyogenes</i> from wound cultures were identified from ward A. 2. New cases of <i>S. pyogenes</i> were identified from 4 other wards (Wards B, D, E, and F), 3. Infection prevention and control measures were implemented as detailed in 	<p>WGS results:</p> <ol style="list-style-type: none"> 1. All isolates from ward A and one isolate from ward D were genetically linked to Cluster 1. 2. All other <i>S. pyogenes</i> were genetically diverse, ruling out a hospital-wide clonal outbreak (Figure 1). 3. The two staff members' <i>S. pyogenes</i> 	<ol style="list-style-type: none"> 1. WGS results were available in a timely manner and prevented the use of institution-wide antibiotics treatment. 2. Detailed and targeted epidemiological

	<p>supplementary Table 1 and Figure 1.</p> <p>4. Oropharyngeal carriage of <i>S. pyogenes</i> was identified in two staff at the institution. The staff were taken off clinical duty and referred for eradication therapy.</p> <p><i>Pre-WGS hypotheses and planning parameters:</i></p> <p>1. The outbreak control team was concerned that this might be an institution-wide clonal outbreak of <i>S. pyogenes</i> and recommended WGS of all isolates until the outbreak settles.</p> <p>2. The outbreak team was concerned if this might be a scenario of staff-to-patient</p>	<p>strains were genetically distinct from all patient isolates ruling out staff-to-patient transmissions (Figure 1).</p> <p><i>Post-WGS conclusions and recommendations by the outbreak control team</i></p> <p>1. Decision was made against prescribing institution-wide antibiotics treatment.</p> <p>2. Additional epidemiological investigation was conducted to study the relationship between the isolate from Ward D and Cluster 1.</p> <p>3. A visiting dermatologist was appointed to review patients on site weekly to</p>	<p>investigation was conducted based on the WGS results.</p> <p>3. Resources were pooled to contain the ongoing transmissions in ward A</p>
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	<p>transmission.</p> <p>3. Possible WGS results and actions considered:</p> <p><i>If WGS shows genetically-linked S. pyogenes cases:</i></p> <ul style="list-style-type: none"> - Hospital-wide antibiotics treatment will be considered to break the chain of transmissions. - Resources will be redeployed to focus more on institution-wide interventions rather than ward-level measures. <p><i>If WGS showed multiple clusters with or without inter-ward genetic linkage</i></p> <ul style="list-style-type: none"> - Environmental hygiene will be further strengthened. - Investigation will be conducted to 	<p>manage their chronic skin conditions.</p> <p>4. Environmental swabbing for <i>S. pyogenes</i> was considered but not done due to limitation in resources and logistical constraints.</p> <p>5. An enhanced environmental cleaning protocol coupled with dermal decolonization of all patients in ward A for 5 days was implemented (supplementary Figure1).</p>	
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	identify the role of the environment as a potential transmission route.		
Phase 3 (5th September - 11th November)	Pre-WGS	Post-WGS	Remarks
Strains sent for WGS: 19 th October 2016 WGS results: 27 th October 2016.	<p>Summary:</p> <ol style="list-style-type: none"> 1. Cases of <i>S. pyogenes</i> were identified in wards A, C, G and H. 2. A general decreasing trend of patients involved in Cluster 1 was noted (Figure 3) 3. By the end of October, the number of patients with skin lesions in Ward A reduced from more than 80% to less than 10%. 	<p>WGS results:</p> <ol style="list-style-type: none"> 1. All isolates from wards A and C were genetically linked to Cluster 1. 2. Isolates from wards G and H were genetically distinct to those from Cluster 1 (Figure 1). 	<ol style="list-style-type: none"> 1. No further cases of genetically linked <i>S. pyogenes</i> transmission were identified in the institution after October 2016.

	<p><i>Pre-WGS hypotheses and planning parameters:</i></p> <ol style="list-style-type: none"> 1. Isolates from Wards A and C are likely to be related to Cluster 1. 2. Isolates from G and H are likely to be genetically diverse (as seen before) 	<p><i>Post-WGS conclusions and recommendations by the outbreak control team</i></p> <ol style="list-style-type: none"> 1. The committee recommended continuing the high level of environmental hygiene. 2. Surveillance for skin lesions in all wards in the institution to be continued. 3. Sporadic cases of <i>S. pyogenes</i> are highly possible in the future however, nosocomial transmission can and should be prevented. 4. To continue genomic surveillance for all <i>S. pyogenes</i> isolates from the institution until December 2016. 	
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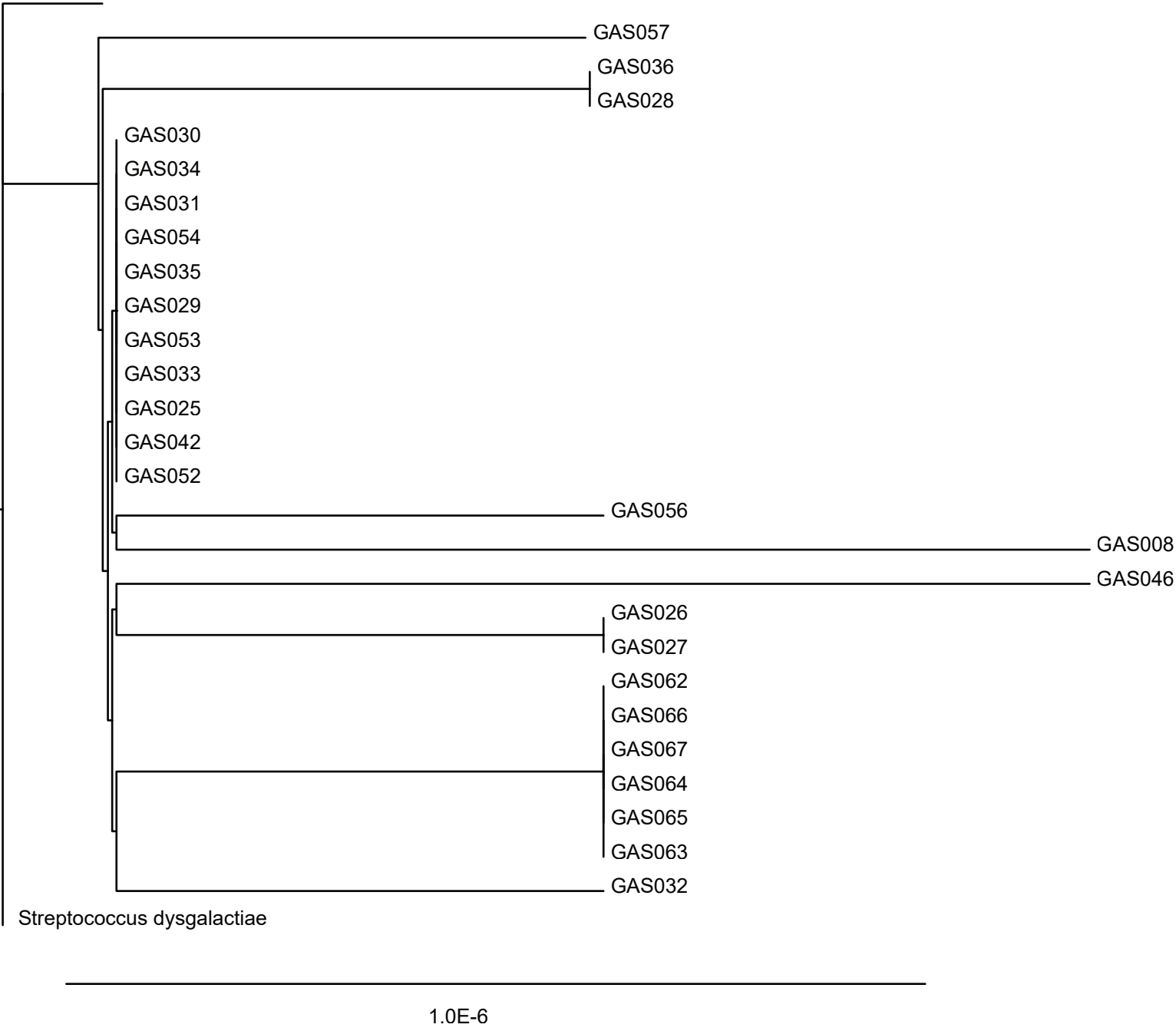
SNP, single nucleotide polymorphism;



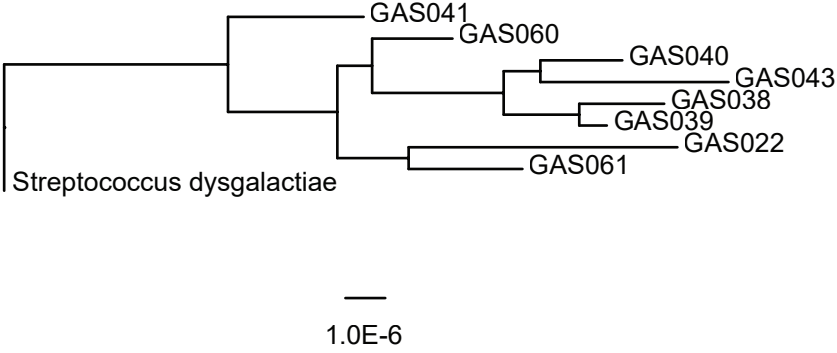
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GAS018	emm81.0	ST-909	01.28.2016	Community
GAS020	emm89.0	ST-101	02.12.2016	Community
GAS010	emm44.0	ST-916	01.17.2016	Community
GAS041	emm11.0	ST-547	07.29.2016	WardB
GAS060	emm11.0	ST-547	09.05.2016	WardG
GAS040	emm11.0	ST-547	07.29.2016	WardB
GAS043	emm11.0	ST-547	07.27.2016	WardB
GAS038	emm11.0	ST-547	07.28.2016	WardB
GAS039	emm11.0	ST-547	07.29.2016	WardB
GAS022	emm11.0	ST-547	03.15.2016	Community
GAS061	emm11.0	ST-547	09.22.2016	WardH
GAS011	emm109.1	ST-633	01.19.2016	Community
GAS055	emm88.3	ST-919	08.16.2016	WardF
GAS058	emm88.3	ST-919	08.29.2016	WardF
GAS024	emm28.0	ST-52	04.30.2016	Community
GAS019	emm113.0	ST-917	01.30.2016	Community
GAS007	emm12.8	ST-36	01.15.2016	Community
GAS045	emm12.0	ST-36	08.02.2016	Staff 2
GAS044	emm12.0	ST-36	07.28.2016	Staff 1
GAS003	emm12.0	ST-36	12.17.2015	Community
GAS005	emm12.7	ST-36	12.17.2015	Community
GAS012	emm12.0	ST-36	01.20.2016	Community
GAS023	emm12.0	ST-36	04.15.2016	Community
GAS047	emm205.0	ST-168	08.08.2016	WardD
GAS002	emm120.0	ST-168	12.09.2015	Community
GAS001	emm120.0	ST-168	11.08.2015	Community
GAS014	emm120.0	ST-168	01.28.2016	Community
GAS050	emm120.0	ST-168	08.10.2016	WardE
GAS051	emm120.0	ST-168	08.12.2016	WardE
GAS059	emm120.0	ST-168	08.31.2016	WardE
GAS009	emm101.0	ST-182	01.18.2016	Community
GAS017	emm80.0	ST-918	02.05.2016	Community
GAS016	emm91.0	ST-13	02.01.2016	Community
GAS015	emm91.0	ST-13	01.31.2016	Community
GAS021	emm91.0	ST-13	02.16.2016	Community
GAS004	emm169.3	ST-53	12.18.2015	Community
GAS006	emm4.0	ST-39	01.04.2016	Community
GAS057	emm4.0	ST-915	08.25.2016	WardA
GAS036	emm4.0	ST-915	06.10.2016	WardA
GAS028	emm4.0	ST-915	06.06.2016	WardA
GAS030 ^{P4}	emm4.0	ST-915	06.09.2016	WardA
GAS034 ^{P2}	emm4.0	ST-915	06.10.2016	WardA
GAS031	emm4.0	ST-915	06.11.2016	WardA
GAS054 ^{P2}	emm4.0	ST-915	08.16.2016	WardA
GAS035	emm4.0	ST-915	06.10.2016	WardA
GAS029	emm4.0	ST-915	06.06.2016	WardA
GAS053 ^{P3}	emm4.0	ST-915	08.15.2016	WardA
GAS033	emm4.0	ST-915	06.10.2016	WardC
GAS025 ^{P3}	emm4.0	ST-915	06.02.2016	WardA
GAS042	emm4.0	ST-915	07.29.2016	WardA
GAS052	emm4.0	ST-915	08.15.2016	WardA
GAS056 ^{P5}	emm4.0	ST-915	08.22.2016	WardA
GAS008	emm4.0	ST-915	01.15.2016	Community
GAS046	emm4.0	ST-915	08.04.2016	WardD
GAS026 ^{P1}	emm4.0	ST-915	06.07.2016	WardA
GAS027 ^{P1}	emm4.0	ST-915	06.08.2016	WardA
GAS062 ^{P4}	emm4.0	ST-915	10.04.2016	WardA
GAS066 ^{P5}	emm4.0	ST-915	10.07.2016	WardA
GAS067	emm4.0	ST-915	10.10.2016	WardC
GAS064 ^{P2}	emm4.0	ST-915	10.07.2016	WardA
GAS065	emm4.0	ST-915	10.07.2016	WardA
GAS063	emm4.0	ST-915	10.06.2016	WardA
GAS032	emm4.0	ST-915	06.10.2016	WardA

Streptococcus dysgalactiae

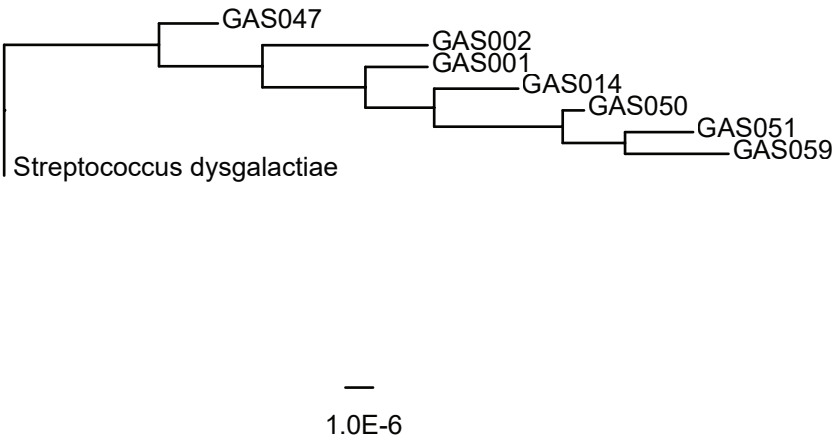
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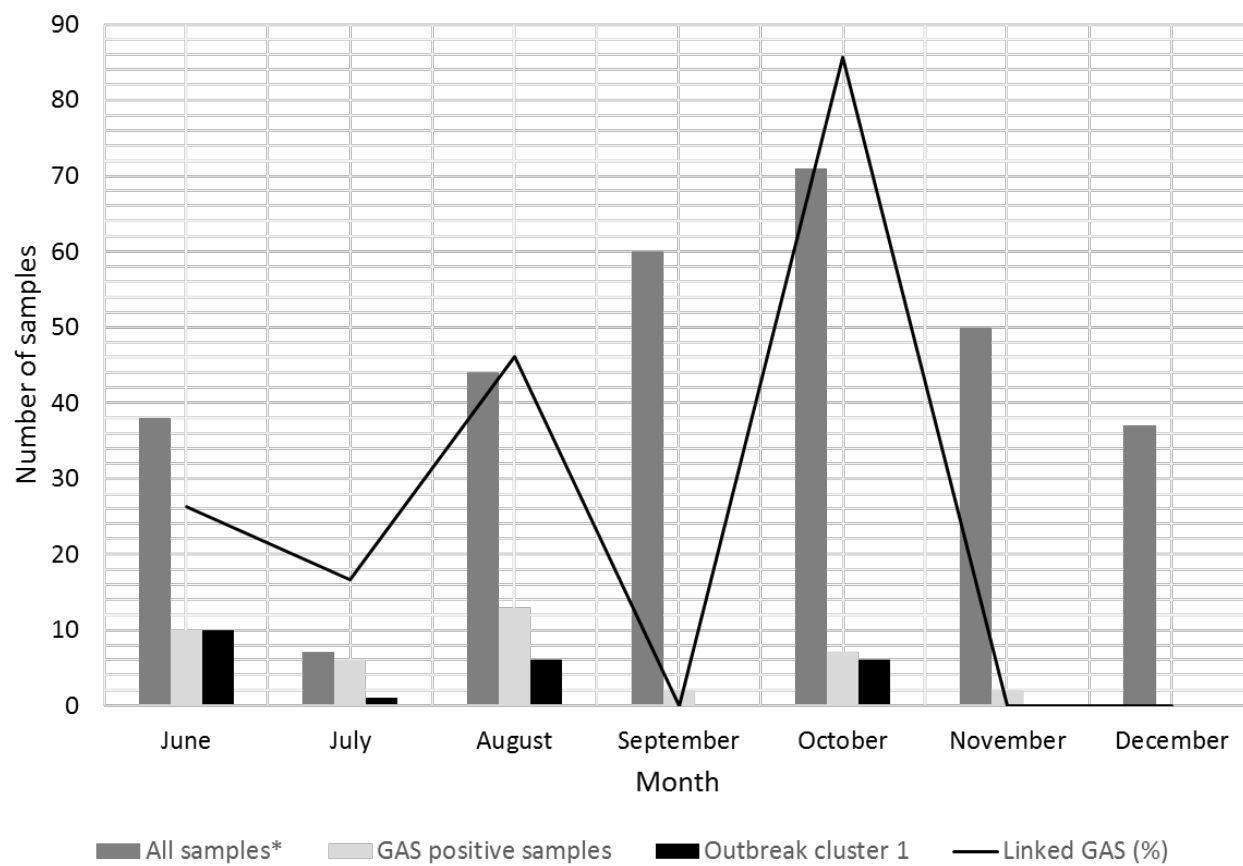


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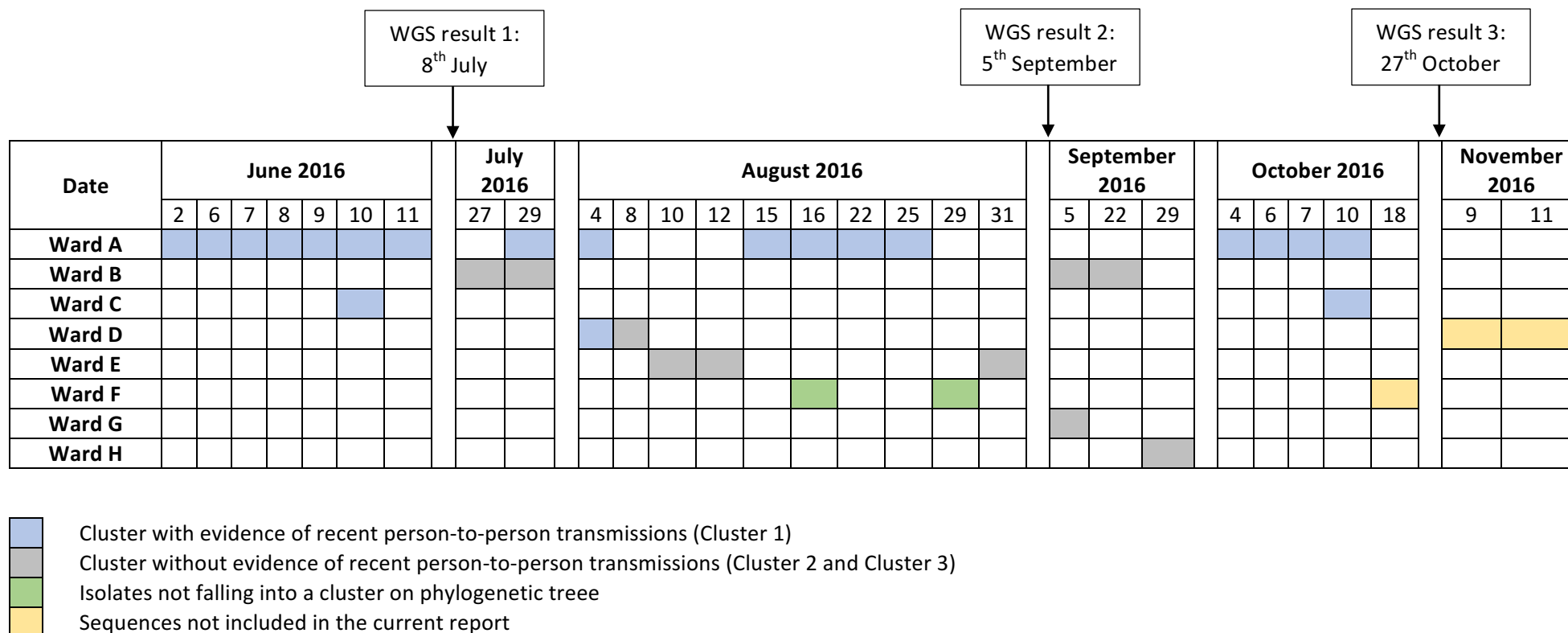
C3





*All samples collected and tested as part of the outbreak investigation

Figure 2: Temporal evolution of the *S. pyogenes* outbreak



WGS, Whole Genome Sequencing

NOTE: Duplicate isolates are not reflected in the table; There may be more than one strains on some dates; Isolates from staff members are not reflected in this figure